CLAIMS

[1] A method for producing a dumbbell-shaped DNA, wherein each of sense and antisense strands is connected at both the 5' and 3' ends of a linear-shaped double stranded DNA by a single stranded DNA of loop structure, comprising the steps of:
1) amplifying a target DNA in a template DNA by PCR using sense and antisense primers, wherein each of the sense and antisense primers contains the following sequence (a) at the 5' end and also contains the following sequences (b), (c), and (d) in order from the 5' end to the 3' end,
(a) a part of a sense sequence of a nickase recognition sequence, comprising the sequence of a region between the site where a nick is introduced by the action of a nickase, and the 3' end,
(b) a sequence capable of forming a loop structure from a single strand,
(c) the entire antisense sequence of the nickase recognition sequence (a),
(d) a sequence complementary to all or part of the sequence of the target DNA;
2) treating the amplified DNA product of step 1) with a nickase of (a);
3) heating and then annealing the nickase treated amplified DNA product of step 2); and
4) treating the heated and annealed amplified DNA product of step 3) with DNA ligase,
wherein the sense and antisense primers used in step 1) are phosphorylated at the 5' end, or the amplified DNA product is phosphorylated at the 5' end after step 1) but before step 4).
[2] A method of CLAIM 1, wherein the dumbbell-shaped DNA is used as a vector for RNA transcription.
[3] A method of CLAIM 1 or 2, wherein the target DNA sequence contains at least one promoter sequence and an siRNA transcription sequence.
[4] A method of CLAIM 3, wherein the dumbbell-shaped DNA is a tandem-shaped siRNA expression vector or a stem loop-shaped siRNA expression vector.
[5] A method of any one of CLAIMS 1~4, wherein the sense primer and/or the antisense primer contains a first spacer sequence and a second spacer sequence, the first spacer and second spacer sequences being complementary to each other, and the first and second spacer sequences being connected so that they are in opposite directions to each other with the sequence (b) interposed.
[6] A method of any one of CLAIMS 1~5, wherein for the sense and antisense primers, the sequence (a) is TN^1AGG (wherein T, A, and G represent thymine, adenine, and guanine, respectively, and N^1 represents any one of adenine, cytosine, guanine, or

thymine), the sequence (b) is (T)n (wherein T represents thymine and n is an integer of at least one), and the sequence (c) is $CCTN^{11}AGC$ (wherein C, T, A, and G represent cytosine, thymine, adenine, and guanine, respectively, and N^{11} represents any one of adenine, cytosine, guanine, or thymine).

[7] A method of CLAIM 6, wherein the sense and antisense primers further contain a first and a second spacer sequence, the first spacer sequence being represented by AG and the second spacer sequence by TC in the sense primer, and the first spacer sequence being represente by TC and the second spacer sequence by AG in the antisense primer, and in the sense and antisense primers, the first and second spacer sequences being connected so that they are in opposite directions to each other with sequence of (b) interposed.

[8] A method of CLAIM 7, wherein the sequence (b) is represented by TTTT in the sequences of the sense and antisense primers.

[9] A method of any one of CLAIMS 1~8, wherein the sense primer and/or the antisense primer is modified by a functional group in at least one position of the nucleic acid backbone or bases of sequence (b) and/or the spacer sequence.

[10] A method of CLAIM 9, further comprising a step of substituting a functional group after step 1).

[11] A composition containing at least one pair of primers consisting of sense and antisense primers, wherein each of the sense and antisense primers followings contains the following sequence (a) at the 5' end and also contains the following sequence (b), (c), and (d) in order from the 5' end to the 3' end,

(a) a part of a sense sequence of a nickase recognition sequence, comprising the sequence of a region between the site where a nick is introduced by the action of a nickase and the 3' end,

(b) a sequence capable of forming a loop structure from a single strand,

(c) the entire antisense sequence of the nickase recognition sequence (a),

(d) a sequence complementary to all or part of the sequence of a target DNA.

[12] A composition of CLAIM 11 for producing a dumbbell-shaped DNA, wherein each of the sense and antisense strands is connected by a single stranded-DNA of loop structure at both the 5' and 3' ends of a linear-shaped double stranded DNA.

[13] A kit for producing a dumbbell-shaped DNA, wherein each of sense and anti-sense strands is connected at both the 5' and 3' ends of a linear-shaped double stranded DNA by a single stranded DNA of loop structure, the kit containing at least one pair of primers consisting of sense and antisense primers, wherein each of the sense and

antisense primers following contains the following sequence (a) at the 5' end and also contains the following sequences (b),
(c), and (d) in order from the 5' end to the 3' end,
(a) a part of a sense sequence of a nickase recognition sequence, comprising the sequence of a region between the site where a nick is introduced by the action of a nickase and the 3' end,
(b) a sequence capable of forming a loop structure from a single strand,
(c) the entire antisense sequence of the nickase recognition sequence (a),
(d) a sequence complementary to all or part of the sequence of a target DNA.

[14] A method for producing a nucleic acid vector, comprising a delivery agent attached to a dumbbell-shaped DNA, wherein each of the sense and antisense strands is connected at both the 5' and 3' ends of a linear-shaped double stranded DNA, by a single stranded DNA of loop structure, comprising the steps of:
1) amplifying a target DNA sequence in a template DNA by PCR using a sense and an antisense primers, wherein each of the sense and antisense primers contains the following sequence (a) at the 5' end and also contains the following sequences (b), (c), and (d) in order from the 5' end to the 3' end,
(a) a part of a sense sequence of a nickase recognition sequence, comprising the sequence of a region between the site where a nick is introduced by the action of a nickase and the 3' end,
(b) a sequence capable of forming a loop structure from single strand,
(c) the entire antisense sequence of the nickase recognition sequence (a),
(d) a sequence complementary to all or part of the sequence of the target DNA;
2) treating the amplified DNA product of step 1) with a nickase of (a);
3) heating and then annealing the nickase treated amplified DNA product of step 2);
4) treating the heated and annealed amplified DNA product of step 3) with DNA ligase; and
5) attaching a delivery agent to a sequence other than the sequence of the target DNA in the DNA ligase treated amplified DNA product of step 4),
wherein the sense and antisense primers used in step 1) are phosphorylated at the 5' end or the amplified DNA product is phosphorylated at the 5' end after step 1) but before step 4).

[15] A dumbbell-shaped DNA produced by a method of any one of CLAIMS 1~ 10.

[16] A dumbbell-shaped DNA, wherein each of sense and antisense strands is connected by a single stranded DNA of loop structure at both the 5' and 3' ends of a linear-shaped

double stranded DNA; containing the following sequences (a') ~ (d'), (a') a part of a sense sequence of a nickase recognition sequence, comprising the sequence of a region between the site where a nick is introduced by the actions of a nickase and the 3' end,
(b') a sequence capable of forming a loop structure from a single strand,
(c') the entire antisense sequence of the nickase recognition sequence (a'),
(d') a target DNA sequence.

[17] A dumbbell-shaped DNA of CLAIM 15 or 16 which can be transfected into cells or tissues so as to express a functional nucleic acid in the cells or tissues.

[18] A dumbbell-shaped DNA of CLAIM 17, wherein the functional nucleic acid to be expressed is a double stranded RNA containing siRNA or a hairpin RNA.

[19] A dumbbell-shaped DNA of CLAIM 17, wherein the functional nucleic acid to be expressed is a ribozyme.

[20] A dumbbell-shaped DNA of CLAIM 17, wherein the functional nucleic acid to be expressed is an antisense RNA.

[21] A dumbbell-shaped DNA of any one of CLAIMS 7~20, which contains all or part of a promoter region transcribed from RNA polymerase III.

[22] A dumbbell-shaped DNA of CLAIM 21, wherein all or part of a promoter region transcribed from RNA polymerase III contains a sequence of 250 bases or less comprising at least one of the following sequences (i)~(iv):
(i) TATA
(ii) CTTACCGTAACTTGAAAGT
(iii) YYTCCCANNRTNCNNYGCRR
(iv) ATGCAAAT or the sequence complementary to the sequence.
(wherein R is either guanine or adenine, Y is either cytosine or thymine, and N is any one of guanine, adenine, cytosine, or thymine).

[23] A dumbbell-shaped DNA of CLAIM 21, wherein all or part of a promoter region transcribed from RNA polymerase III contains a sequence 150 bases or less comprising at least one of the following sequences (i')~(ii'):
(i') RRYNNARYGG
(ii') GGTTCGANTCC
(wherein R is either guanine or adenine, Y is either cytosine or thymine, and N is any one of guanine, adenine, cytosine, or thymine).

[24] A dumbbell-shaped DNA of any one of CLAIMS 21~23 which contains any one of the sequences of SEQ ID NOS: 1, 22, 23, and 25.

[25] A dumbbell-shaped DNA of any one of CLAIMS 7~24, wherein the functional nucleic acid to be expressed is targeted against a gene related to virus or cancer.

[26] A dumbbell-shaped DNA of CLAIM 25, wherein the virus is selected from the group consisting of HIV, HCV, and HBV.

[27] A dumbbell-shaped DNA of CLAIM 15 or 16 which can be transfected into cells or tissues so as to suppress the expression of genes.

[28] A dumbbell-shaped DNA of CLAIM 27, which is DNAzyme.

[29] A dumbbell-shaped DNA of CLAIM 27, which functions as a decoy.

[30] A dumbbell-shaped DNA of any one of CLAIMS 15~29, which is a modified DNA constructed from optically active boranophosphate.

[31] A composition containing a dumbbell-shaped DNA of any one of CLAIMS 15~30.

[32] A pharmaceutical composition containing a dumbbell-shaped DNA of any one of CLAIMS 15~30.